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CHARACTERIZATION OF BILATERAL TRIGEMINAL CONSTRICTION INJURY USING AN OPERANT FACIAL PAIN ASSAY

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Abstract—In order to better understand and treat neuropathic pain, scientific study must use methods that can assess pain processing at the cortical level where pain is truly perceived. Operant behavior paradigms can accomplish this. We used an operant task to evaluate changes following chronic constriction injury to the trigeminal nerves. We also relate these behavioral changes to immunohistochemistry of transient receptor potential channels vanilloid 1 and melastatin 8 (TRPV1 and TRPM8) in the trigeminal ganglia. Following nerve injury, successful performance of the operant task was reduced and aversive behaviors were observed with 10 and 37 °C stimulation, indicating cold allodynia and mechanical allodynia respectively. In contrast, while aversive behaviors were observed with 48 °C stimulation, successful performance of the operant task was not substantially hindered following injury. These behavioral changes were accompanied by an increase in TRPV1 positive cells and an increased intensity of TRPM8 staining at 2 weeks post-injury, when cold allodynia is maximal. These findings suggest that the incorporation of operant behavioral assessment in the study of pain may provide insight into the relationship among peripheral changes, motivational drive, and pain. Understanding this relationship will allow us to better treat and prevent chronic neuropathic pain.

Key words: trigeminal neuropathic pain, operant, TRPM8, cortical processing, allodynia, TRPV1.

INTRODUCTION

Chronic pain in the orofacial region is experienced by 25% of the United States, including disorders such as trigeminal neuralgia and pain associated with trauma to the face and mouth (Lipton et al., 1993). However, the mechanisms underlying these conditions are not completely understood, particularly with respect to neuropathic pain. To fully understand trigeminal neuropathic pain it is important to utilize models and behavioral assessment that directly impact and measure the trigeminal system, as differences have been noted between neuropathic injury models affecting extracephalic and cephalic sites (Benoliel et al., 2001; Latrémolière et al., 2008; Kayserl et al., 2011). Furthermore, it has become increasingly evident that chronic, maladaptive pain is maintained by changes at the cortical level (de Leeuw et al., 2005; Seifert and Maihofner, 2009). Therefore, the evaluation of trigeminal pain and analgesia in experimental animals should incorporate methods that directly evaluate cortical processing by requiring that the animal make a decision about its environment.

Operant assays directly evaluate cortical processing of nociceptive input (Vierck et al., 2008). Additionally, operant tests more closely resemble functional assessments that are made clinically. That is, operant tests ''ask'' the animal subject if the pain they are experiencing hinders them from performing functionally important or rewarding tasks. More groups are beginning to use operant methods to evaluate thermal allodynia and hyperalgesia following sciatic or spinal nerve injury (Vierck et al., 2005; Jabakhanji et al., 2006; Walczak and Beaulieu, 2006). However, operant assessment of thermal hyperalgesia following trigeminal nerve injury is currently limited to one publication (Kumada et al., 2012).

In this study, we sought initially to determine the effects of bilateral chronic constriction injury (CCI) of the infraorbital trigeminal nerves on thermal sensitivity in the face, using an operant assay that we have previously characterized (Neubert et al., 2005, 2006; Rossi et al., 2006). We assessed responses to cold, hot, and neutral stimulation before and up to 4 weeks after surgery. The thermode set at the neutral (body) temperature may be a surrogate for mechanical sensitivity. In addition to operant assessment, we also used a behavioral scoring system concurrent with operant testing to assess changes in aversive behaviors exhibited with stimulation. These behavioral assessments were also accompanied by assessment of nerve inflammation and injury and

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Abbreviations: ANOVA, analyses of variance; CCI, chronic constriction injury; PBS, phosphate-buffered saline; TRPM8, transient receptor potential channel melastatin 8; TRPV1, transient receptor potential channel vanilloid 1.

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immunohistochemistry against the transient receptor potential (TRP) channels vanilloid 1 and melastatin 8 (TRPV1 and TRPM8, respectively) in trigeminal ganglia from sham- and CCI-treated rats. Changes in TRP channel expression, particularly TRPV1 (Christoph et al., 2006), have been implicated in peripheral sensitization that contributes to neuropathic pain, and evidence also suggests that TRPM8 may play a role as well (Xing et al., 2006, 2007; Su et al., 2012). The incorporation of operant behavioral assessments may allow us to better understand the relationship between processes involved in establishing neuropathic pain states and changes in motivation and behavior that impact pain. Understanding this relationship is critical for improving treatment and developing ways to prevent neuropathic pain.

EXPERIMENTAL PROCEDURES

Animals

Male hairless Sprague–Dawley rats (n = 45, 5–7 weeks old, Charles River, Raleigh, NC) were housed in groups of four in enriched housing (see (Rossi and Neubert, 2008) for description of enrichment). All rats were maintained in a standard 12-h light/dark cycle and were allowed access to food and water *ad libitum* when not being tested. Rats' weights were recorded every week to monitor general health. Animal testing procedures and general handling complied with the ethical guidelines and standards established by the Institutional Animal Care & Use Committee at the University of Florida (Institute of Laboratory Animal Resources, 1996).

Induction of neuropathic pain

Following training and baseline behavioral testing, rats received either a bilateral CCI or sham operation of the infraorbital portion of the trigeminal nerve (maxillary division). The operant chambers are designed to stimulate both sides of the face simultaneously, so an ipsilateral versus contralateral comparison would be difficult to make. This is why we opted to perform a bilateral injury. These surgeries were performed intraorally, as described by Imamura et al. (1997), with some modification. We chose this intraoral route rather than an external method so that the incision site would not be within the field of thermal testing. Briefly, rats were deeply anesthetized with a ketamine/xylazine cocktail (2:1, 1.2 mg/kg). For local anesthesia and hemostasis, xylocaine (2%, 1:100,000 epinephrine) was applied to the intraoral incision area. An incision was made beginning from the hard palate and extending approximately 1 cm anteriorly toward the maxillary incisors, roughly parallel with the lip. The nerve was exposed from surrounding tissues by blunt dissection and gently elevated with a hooked instrument so that two ligatures (5–0 vicryl suture) could be tied securely around the nerve. This procedure was the same for sham surgeries, except that sutures were passed under the nerve, but not tied. This process was repeated for the second side. All incisions were closed with 2–4 ligatures (5–0 vicryl suture). All surgeries were performed by the same investigator and nerves were treated in the same order each time. Rats' weights, general behavior, and facial scratching were tracked for 1 week after the surgeries to monitor healing and recovery. A blinded observer noted the location and extent of scratching within the innervated area. Behavioral testing began after the one-week recovery period. Three sets of surgeries were conducted, for a total of 18–22 rats assigned to each treatment.

BEHAVIORAL TESTING

Operant evaluation of thermal sensitivity

Facial testing was conducted using a reward-conflict operant testing paradigm as described previously (Neubert et al., 2005). In this paradigm, the rat may choose to experience thermal stimulation that may be painful in order to obtain a milk reward, or they may abstain from both reward and experiencing pain. Briefly, rats were trained to drink sweetened condensed milk while making facial contact with a single thermode and lick-tube. During the training period (approximately 2 weeks) baseline intake was recorded, and rats were considered ready for experimental testing once their average baseline intake was 10 g or greater at 37 °C. The facial testing region for each animal was depilitated under light isoflurane anesthesia (inhalation, 2.5%) once a week to maximize thermal stimulus contact. Rats were fasted for 13–15 h prior to stimulus testing, except for 37 °C.

Following training, baseline data were recorded twice for each stimulus temperature (10, 37, and 48 °C). For every 20-min testing session, three outcome measures were calculated for each rat as described previously (Neubert et al., 2005): number of licks, number of stimulus contacts, and the pain index, which is the number of licks (successful attempts) divided by the number of stimulus contacts (total attempts). Following surgical treatment and recovery, rats were tested with one of the following schedules: three times at 10 °C and once at 37 °C per week; twice at 10 °C, once at 37 °C, and once at 48 °C per week; or once each at 10, 37, and 48 °C per week. We were initially interested in characterizing cold allodynia, as this is of more clinical relevance than heat hyperalgesia. In subsequent groups of rats we included a hot stimulus to determine if heat hyperalgesia was also present. It should be noted that rats were fasted prior to each stimulus, except for 37 °C, with no more than three overnight fasts per week.

Evaluation of aversive behaviors during operant testing

During the first cycle of testing it was noted that some rats would tilt their heads in an effort to displace the stimulus away from the portion of the face innervated by the infraorbital nerve (Fig. 1, and Supplemental video). The experimenter who made this observation was blinded to treatment. The rats exhibiting this behavior were later identified as CCI-treated animals. Thus for subsequent groups of surgically treated animals, a system of scoring was established to assess additional innate and aversive behaviors directed toward the stimulus in addition to changes in operant outcomes. Rats were observed during the first 5 min of operant testing and one point was assigned for the presence of three aversive behaviors (head tilting, wiping or pushing at the stimulus with forepaws, and biting at the stimulus). We chose to assess the first 5 min of testing rather than the entire testing period because untreated rats attend to operant task completion immediately and typically do